Multimodal Advanced Microscopy to Study Drug Responses in Complex Cell Models

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Abstract

- More translational models are needed for new medicines. iPSC derived 3D models may provide more benefits than current 2D approaches
- These complex models must be characterised and validated
- MDC has developed multimodal advanced microscopy approach to combine high-resolution microscopy with complex cell model development
- Using combined high resolution confocal microscopy and high content spinning disk we can determine drug action and deconvolute targets. We can support this with bespoke image analysis workflows for validation and quantification of drug response
- Here we show two models in development at MDC, a CNS triculture model for studying CNS in neuroinflammation and disease, and a CMEF cardiac model, a 3D model for assessing cardiotoxicity

Combined High Resolution Confocal Microscopy of CNS iPSC Tricultures for Drug Discovery

3D imaging characterisation of tricultures of iPSC-derived neurons, astrocytes and microglia

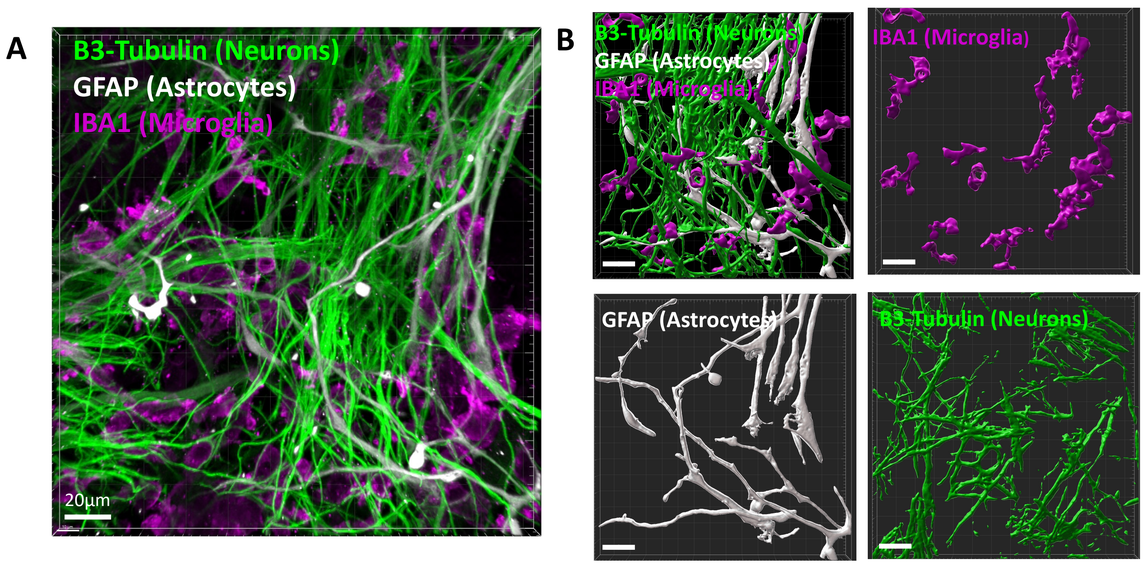

Figure 1. Detailed 3D imaging has shown ultrastructural arrangement of CNS tricultures, showing a network of neurons, astrocytes and microglia. **A.** Representative high resolution laser scanning confocal of iPSC-derived triculture showing neurons, microglia and astrocytes. **B.** 3D rendering of tricultures allows for assessment of cell morphology to show cell morphology/phenotypic modulation in response to therapeutics or inflammation/ disease stimuli. Use of specialised 3D analysis software can be applied to attain detailed measurements of cell shape, length and branching.

Live antibody binding screen assay established in CNS iPSC-derived tricultures

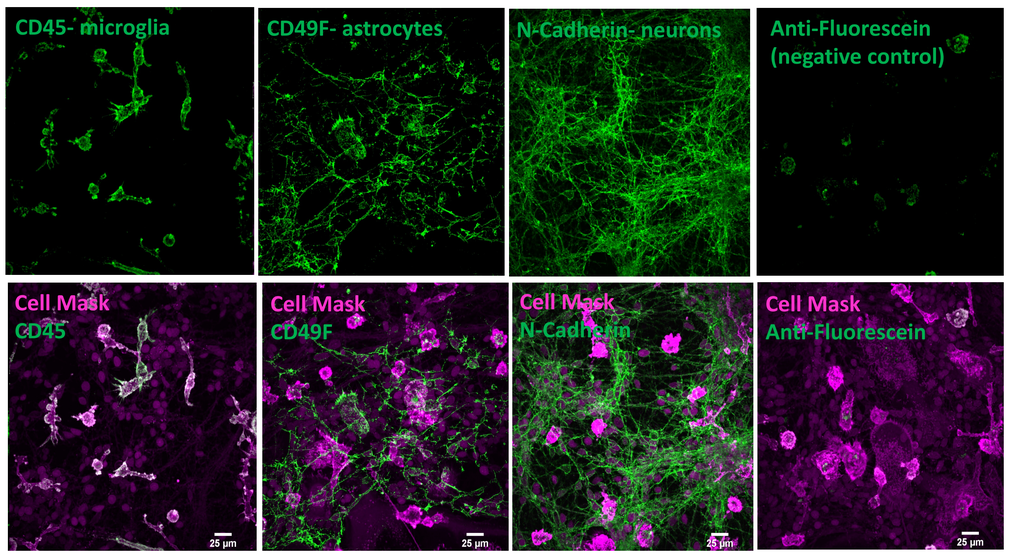

Figure 2. Tricultures were imaged using a spinning disk confocal high content system. Shown are control antibodies binding the extracellular domain of membrane proteins expressed in each of the 3 cell types (microglia, astrocytes and neurons). Anti-fluorescein antibody labelling is shown as a negative control. High content imaging analysis software was used to build up imaging analysis pipelines, assessing the fluorescence intensity of the antibodies.

3D Imaging of CMEF Cardiac Model for Assessing Cardiotoxicity

Characterisation of Cardiac CMEF Model

iPSC cardiomyocytes (CM), primary cardiac endothelial cells (E) and cardiac fibroblasts (F) are combined in a physiological cell ratio to create spheroids that spontaneously beat.

Figure 3 Representative high resolution 3D images to visualise the Cardiac CMEF model **A. The** three cell types were identified by cell specific immunostaining. Cardiomyocytes with Alpha-actinin1, endothelial cells with CD31 and fibroblasts with Collagen1**. B.** Intra-spheroid formation of the endothelial component (CD31) can be visualised over time to show how the cells come together to form structures.

Figure 4 Genetically edited cells expressing zsGreen endothelial and mCherry fibroblast enable direct live visualisation of cells and spheroid vasculature.

Interrogate Drug Response by Structural and Functional Assays

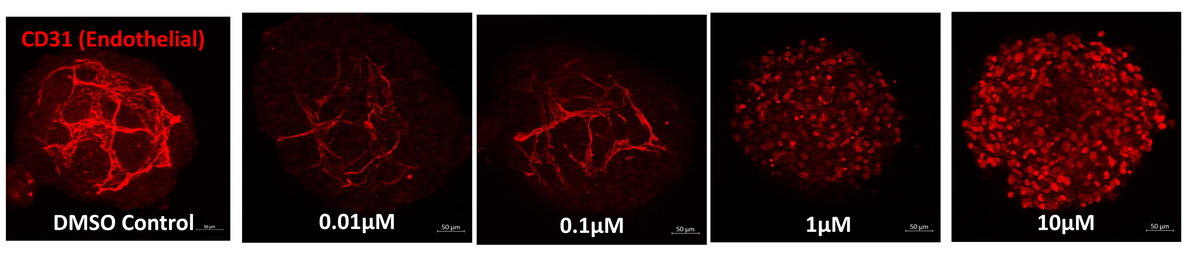

Figure 4 Treatment of spheroids with doxorubicin (DOX). At Day 5 spheroids were treated for 72 hours with a range of DOX concentrations, then fixed and stained with CD31. Disruption of the cardiac spheroid vasculature can be quantified by 3D image analysis.

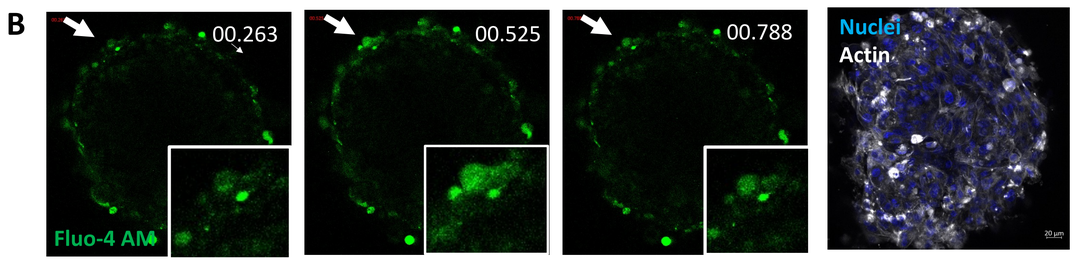

Figure 5 Live imaging of CMEF spheroids to measure physiological responses. Left, calcium fluctuations could be visualised around the spheroid edge using fast frame imaging, bursts of calcium were seen as the cell contracted over a couple of seconds. Right, live nuclei and actin stains can also be used on the spheroid and using fast frame imaging, the contraction of the spheroid and cell displacement can be observed.

Conclusions **Combining high resolution microscopy capabilities with complex cell models at MDC provides powerful assays to answer relevant questions within drug discovery.** For both models, the increase in complexity and innovative imaging analysis may demonstrate their improved utility for drug screening or mechanism of action studies.

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